

## POSTER PRESENTATIONS

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## Comparison of an excision and a sponge sampling method for measuring salmonella contamination of pig carcasses

PD 01

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**Summary:** The aim of this study was to determine if an excision sampling method and a sponge sampling method give comparable results when trying to isolate salmonella from pig carcasses. During ten sampling days in one abattoir in total 312 carcasses were sampled; each carcass was sampled with both sampling methods to get paired observations. The number of salmonella positive excision samples (31 of 312) was significantly higher ( $P=0.00013$ ) than the number of salmonella positive sponge samples (9 of 312). Sensitivity of the sponge method compared to the excision method was 6.5% and the comparability of both tests was low (kappa value was 0.08). As it seems that salmonella contamination levels of fresh pork are highly underestimated with the actually used sampling methods, the authors recommend that EU-authorities prescribe a destructive salmonella test for monitoring pig carcasses after slaughter in all EU-countries or a swab/sponge method with a comparable sensitivity.

**Introduction :** In order to monitor the product of pig abattoirs, samples for bacteriological investigation can be taken of the end product of the abattoirs: split pig carcasses. Several methods for carcass sampling have been described (Gill and Jones, 2000, Snijders et al., 1984, Dorsa et al., 1996, Dorsa et al., 1997, Swanenburg et al., 2000). In general these methods can be divided in destructive methods, such as the cork borer method, and non-destructive methods, such as swabs, sponges and contact plates. Dutch abattoirs have to sample their carcasses regularly for the presence of salmonella to get permission to export their products to the USA. The sampling method is prescribed exactly by the American Food Safety and Inspection Service and is carried out by (or under supervision of) the State Veterinary Inspections Service for Livestock and Meat (Anonymous, 2002). On the other hand, abattoirs can use their own sampling schemes and methods for their own monitoring purposes. In this study an excision sampling method by which a piece of belly hide of the carcass was cut out was compared with the sponge sampling method, which is used for the "USA" sampling of carcasses. Aim of this study was to determine if these two sampling methods give comparable results when isolating salmonella from pig carcasses.

**Materials and Methods:** Samples were collected in a Dutch pig abattoir during five weeks on Tuesdays and Thursdays. On each sampling day two different samples were collected from approximately 40 randomly chosen carcasses. The excision sample was taken from carcasses, hanging from the hind legs in the line, just after veterinary inspection. The sample consisted of approximately 60 cm<sup>2</sup> of belly hide, cut from the cutting surface of the belly just above the sternum (the cutting surface of the belly originates from the opening of the belly by the belly opening saw). The contamination of this surface represents the contamination caused by machinery in the slaughterline. The sample was cut from the carcass using a sterile meat knife and a pair of tweezers and put in a sterile plastic stomacher bag. The carcass was identified to ensure that the sponge sample would be taken from the same carcass. Sponge samples were taken 24 hours after slaughter from the same carcasses as the excision samples. For the sponge sample the Meat Turkey Carcass Sampling Kit (Nasco, USA) was used. The samples were taken according to the official instructions laid down by the Dutch State Veterinary Inspections Service for Livestock and Meat (RVV) (Anonymous, 2002), which are based on the rules of the Food Safety and Inspection Service (USA, FSIS, 1996). Three areas of 100 cm<sup>2</sup> each were swabbed, one area on the belly 10 cm from the cutting surface, one area on the ham (both with one side of the sponge), and one on the jowl of the pig (with the other side of the sponge). Sponges were put in the plastic bag (Whirl-Pak) and transported to the laboratory. Salmonella was isolated from the samples according to standard procedures (Buffered Peptone Water, Tetrathionate broth, Brilliant Green Agar, confirmation with Triple Sugar Iron agar, Lysine Decarboxylase and urea-agar). Salmonella isolates were serotyped with group A-G anti-salmonella serum. Statistical analysis of data was done with Statistix 7.0.

**Results:** A total of 312 paired observations for both tests were made. Salmonella isolation results for both sampling methods are presented in table 1. The number of salmonella positive excision samples was significantly higher than the number of positive sponge samples (McNemar Chi square test, P=0.00013). Using the destructive sample as golden standard the sponge method had a sensitivity of 6.5% compared to the excision method. The kappa value for comparability of the two sampling methods was 0.08, where a kappa value of 0.4 is considered as a reasonable and a kappa value of 0.6 as a good comparability between tests.

**Table 1.** Results of salmonella isolation from sponge samples and excision samples taken from 312 pig carcasses.

		Result excision sample		Total	
		Positive	Negative		
Result sponge sample	Positive	2	4	6	(1,9%)
	Negative	29	277	306	
Total		31	281	312	

**Discussion:** Considerably more excision samples than sponge samples were salmonella positive. It would have been logical to expect more positive results with the sponge method than with the excision method because with the sponge method a larger area was sampled and from more different locations of the carcass. On the other hand, sponge sampling was carried out 24 hours after slaughter, after the carcass had been chilled and cooled. During this time span, salmonella can attach itself to the skin or withdraw in hair follicles (Berends, 1998), and will not be removed by sponging. Furthermore, although not investigated in this study, the cutting edge of the carcass is probably more contaminated than skin surfaces as a result of contamination by contact with contaminated machinery during the slaughter process. Swanenburg et al. (2001) showed that contamination of slaughterhouse machinery during the slaughter process is largely responsible for the contamination of pig carcasses with salmonella. Although in total salmonella was isolated from more carcasses with the excision method than with the sponge method, 4 carcasses were positive when sampled with the sponge method, which were negative with the excision method. This can be explained by the fact that different locations on the carcass were sampled with both methods and at different points in time.

The results of this study show that using the internationally recognised USA sponge sampling method results in a serious underestimation of the carcass contamination in pig abattoirs. If prevalence data of carcasses, obtained with this method, are published or are used to show that the salmonella "problem" is not very large and with that suggesting that the situation is under control, this will give a false sense of security to abattoir personnel and management, customers, consumers and authorities. In our opinion more sensitive methods, as for example as described here, are more appropriate to give a clear picture of the real situation in abattoirs. Also the more detailed information gives better opportunities to focus intervention strategies more precisely and leaves more room for improvement than the very low prevalence found with (variations on) the USA-sponge-method (S. rensen et al, 2001). An advantage of the USA sponge method is that it is well described and internationally accepted in the industry. To introduce a new and much more sensitive sampling method which is comparable between studies and countries requires international agreement on the method and support from the authorities. EU-wide and preferably world wide agreement and implementation on a sensitive sampling method will make it possible to compare salmonella prevalences of carcasses between countries and pork producers.

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## PD 02

### Evaluation of Pooled Serum and 'Meat-Juice' in a *Salmonella* ELISA for Pig Herds

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**Summary:** Samples of 'meat-juice', serum, caecal contents and carcass swabs from 420 pigs from 20 finishing farms were tested for *Salmonella* bacteriologically and serologically by ELISA on individual samples or on pools of serum or meat juice. In addition pooled floor faeces were taken from the finishing pens on the farm of origin.

*Salmonella* was isolated in samples from 19 of the 20 farms. 32.8 % of pooled pen faeces and 24.3 % of caecal samples were positive but *Salmonella* was only found in 1.7 % of carcass swabs. 43.2 % of individual 'meat-juice' samples and 25.3 % of serum samples gave positive ELISA results. Neither the individual or pooled ELISA tests showed a statistically significant correlation with caecal carriage of *Salmonella* or contamination of carcasses, although the percentage of positive pen faecal samples did correlate significantly with caecal positives. Only serum mean optimal density from pools of 5, 10 or 20 sera correlated significantly with *Salmonella* prevalence in pen faecal samples but all pooled serum and meat-juice optimal density or sample/positive ratios correlated significantly with the percentage individual ELISA positives. This suggests that pooled serum or meat-juice could be used as an alternative to individual samples for ranking herds.

**Keywords:** serology, monitoring, comparison, swine, contamination

**Introduction:** Monitoring for *Salmonella* in slaughter pigs is important to enable targeted control measures to be applied on significantly infected farms and at the abattoir. Serological testing using a LPS based mix ELISA has been shown to be suitable for ranking herds according to likely weight of infection (Nielsen et al., 1998), but testing sufficient numbers of samples to obtain an accurate herd ranking is expensive for an industry in severe financial difficulties. Pooled samples are routinely used to maximise bacteriological detection of *Salmonella* with limited resources. This paper describes a study designed to assess the suitability of pooling of serum or meat juice samples for ranking the *Salmonella* status of pig herds.

**Materials and Methods:** Approximately 420 serum, meat juice, carcass swab, and caecal contents samples were obtained from groups of slaughter pigs from 20 farms. Carcass swabs were taken according to a US/Danish protocol. In addition, the farms were visited on the day before slaughter and pooled faeces collected from pens occupied by the pigs to be slaughtered. Serum and meat juice samples were tested by ELISA (Vetsign *Salmonella* ELISA Kit; Guildhay) as individual samples and as pools comprising 5, 10 and 20 individual subsamples. Bacteriological culture carried out by 18 hours pre-enrichment at 37 °C Buffered Peptone Water (BPW; Merck), 48 hours selective enrichment at 41.5 °C in DIASALM medium (Merck), with subculturing on to Rambach agar (Merck) plates after 24 and 48 hours culture. The plates